PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 005092.00061	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US04/00630	International filing date (*day/month/year*) 12 January 2004 (12.01.2004)	(Earliest) Priority Date (*day/month/year*) 15 January 2003 (15.01.2003)
Applicant PROTASIS CORPORATION		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the Report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:
   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.
2. ☐ **Certain claims were found unsearchable** (See Box I).
3. ☐ **Unity of invention is lacking** (See Box II).
4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 23
   ☒ as suggested by the applicant.
   ☐ None of the figures
   ☐ because the applicant failed to suggest a figure.
   ☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/00630

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 27/26, 27/447
US CL : 204/450, 518, 600, 627

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 204/450, 518, 600, 627, 543, 409

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN Caplus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,298,143 B1 (IVORY et al) 29 March 1994 (29.03.1994), Entire document	2, 72-74 --- 1, 3-19, 43-71, and 75-97
X --- Y	US 6,277,258 B1 (IVORY et al) 21 August 2001 (21.08.2001) Entire document.	3 and 15 --- 1, 2, 4-19, 43-97
X --- Y	KOEGLER, W.S. and C.F. Ivory, Field Gradient Focusing: A Novel Method For Protein Separation, Biotechnol. Prog. 12, 822-836. (1996) Especially p. 828-829, Equipment section.	80-82 --- 4, 16, 55-59, 83
Y	US 4,680,102 A (ISHIWATARI) 14 July 1987 (14.07.1987), Especially Figure 1 and Column 2, lines 56-66.	1, 6-12, 14, 17-19, 43-46, 68-71
Y	US 2001/0034435 A1 (NOCHUMSON et al) 25 October 2001 (25.10.2001), Especially Figure 1 and Paragraph 0060.	17 and 18
Y	US 6,284,115 (APFFEL) 04 September 2001 (04.09.2001), Especially Figure 1; Column 6, line 62 - Column 7, line 12	19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
02 August 2004 (02.08.2004)

Date of mailing of the international search report
01 AUG 2004

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Form PCT/ISA/210 (second sheet) (July 1998)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
PETER D. MCDERMOTT
BANNER & WITCOFF, LTD
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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*) 31 AUG 2004

Applicant's or agent's file reference
005092.00061

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/00630	12 January 2004 (12.01.2004)	15 January 2003 (15.01.2003)

International Patent Classification (IPC) or both national classification and IPC
IPC(7): G01N 27/26, 27/447 and US Cl.: 204/450, 518, 600, 627

Applicant
PROTASIS CORPORATION

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☒ Box No. VII — Certain defects in the international application
- ☒ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/00630

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ in written format

      ☐ in computer readable form

   c. time of filing/furnishing

      ☐ contained in international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/00630

Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1, 4-14, 16-71, 75-79, and 83-97	YES
	Claims	2, 3, 15, 72-74, and 80-82	NO
Inventive step (IS)	Claims	20-42	YES
	Claims	1-19, 43-97	NO
Industrial applicability (IA)	Claims	1-97	YES
	Claims	NONE	NO

2. Citations and explanations:

Please See Continuation Sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US04/00630

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

In claim 84, at line 7, applicant's attention is brought to the limitation that these first electrodes establish an electric field gradient in the titration chamber. Given later limitations to second electrodes repeating this function and the general structure of these devices, it seems that the gradient might be intended to be established in the separation chamber.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US04/00630

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

Claims 68-83 are objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: the limitation to an electrode chamber having "substantially uniform depth and non-uniform width" is recited twice in each of the independent claims (68, 72, 76, and 80).

Claims 86, 87, and both claims 85 are objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: the claims are not numbered consecutively with Arabic numerals, and the presence of two claims numbered 85 renders claims 86 and 87 indefinite. Herein, the first listed claim 85 is referenced as 85a and the second as 85b. Claims 86 and 87 are treated as being dependent on claim 85a, because it avoids lack of antecedent basis for the second electrode chamber.

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:

Claims 2 and 72-74 lack novelty under PCT Article 33(2) as being anticipated by Ivory et al (US 5,298,143).

Addressing claim 2, Ivory et al disclose a device suitable for determining the isoelectric point of a charged analyte (Figure 16), comprising: a chamber (53) comprising liquid inlets and outlets (86, 89, 321-345), an electrode array (98, 422-424) isolated from the chamber and operative to establish an electric field gradient in the chamber, and a reservoir in fluid communication with the chamber. (Reservoir 135, Figure 12; Column 13, lines 12-23) Components of the system can be termed "titration" chamber and reservoir, if used for a titration method.

Addressing claim 72, Ivory et al disclose a device suitable for determining the isoelectric point of a charged analyte (Figure 20), comprising: a chamber (653) comprising liquid inlets and outlets (454, 456, and 458), an electrode chamber (650) that has a substantially uniform depth and non-uniform width, two electrodes (98) in the electrode chamber operative to establish an electric field gradient in the chamber, and a reservoir in fluid communication with the chamber. (Reservoir 135, Figure 12; Column 13, lines 12-23) Components of the system can be termed "titration" chamber and reservoir, if used for a titration method.

Addressing claim 73, Ivory et al disclose separation of the titration and electrode chambers by a permeable material. (Membrane 660)

Addressing claim 74, Ivory et al disclose the titration chamber having a uniform cross-section flow channel. (Figure 20, Membrane 660; Column 10, lines 33-37)

Claims 3 and 15 lack novelty under PCT Article 33(2) as being anticipated by Ivory et al (US 6,277,258).

Addressing claim 3, Ivory et al disclose a device suitable for determining the isoelectric point of a charged analyte (Figures 3-6), comprising: a chamber (12) comprising liquid inlets and outlets (114 and 116), an electrode array (22) isolated from the chamber and operative to establish an electric field gradient in the chamber, an analyte band detector operative to generate positional signals corresponding to positions of a band of analyte in the titration chamber (Column 3, lines 32-37; Column 7, lines 8-12), and a processor operative to receive positional signals from the analyte band detector and to determine the magnitude of position change of an analyte band in the titration chamber in the course of titration. (Column 3, lines 32-37; Column 7, lines 8-12) The chamber can be termed a "titration" chamber, if used for a titration method.

Addressing claim 15, Ivory et al disclose the use of a potentiometric detector. (Column 7, lines 9-10)

Claims 80-82 lack novelty under PCT Article 33(2) as being anticipated by Koegler et al.

Addressing claim 80, Koegler et al disclose a device suitable for determining the isoelectric point of a charged analyte (Figure 7, Equipment section), comprising: a titration chamber comprising liquid inlets and outlets, an electrode chamber with uniform depth and non-uniform width, two electrodes in the electrode chamber operative to establish an electric field gradient in the titration chamber, and an analyte band detector operative to generate positional signals corresponding to positions of a band of analyte downstream of an electric field gradient in the titration chamber. Components of the system can be termed "titration" chamber and reservoir, if used for a titration method.

Addressing claim 81, Koegler et al disclose the titration and electrode chambers being separated by a permeable material

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

(Dialysis tubing, 1st paragraph of Equipment section)

Addressing claim 82, Koegler et al disclose the titration channel having a uniform cross-section (6.4 mm diameter tubing, constant diameter illustrated in Figure 7)

Claims 1, 9,14, and 68-70 lack inventive steps under PCT Article 33(3) as being obvious over Ivory et al (US 5,298,143) in view of Ishiwatari.

Relevant to claims 1, 9, and 14, Ivory et al disclose a device suitable for determining the isoelectric point of a charged analyte (Figure 16), comprising: a chamber (53) comprising liquid inlets and outlets (86, 89, 321-345), an electrode array (98, 422-424) isolated from the chamber and operative to establish an electric field gradient in the chamber, and a reservoir in fluid communication with the chamber. (Reservoir 135, Figure 12; Column 13, lines 12-23) Components of the system can be termed "titration" chamber and reservoir, if used for a titration method.

Relevant to claims 68-70, Ivory et al also disclose an electrode chamber with uniform depth and non-uniform width (Chamber 650, Figure 20), with at least two electrodes (98) in the chamber operative to establish an electric field gradient in the titration chamber (653). This embodiment also provides a permeable barrier between the titration and electrode chambers (membrane 660), and a titration chamber with a uniform cross-section (Figure 20, tubular membrane)

Ivory et al do not explicitly disclose the use of a pH sensor positioned for exposure to the liquid introduced into the titration chamber (Claim 1), where the pH sensor comprises an ion-selective electrode. (Claim 14)

Ishiwatari discloses an electrophoretic device that uses pH sensors (8a and 8b, Figure 1) to detect the pH of the buffer solution that is used in the electrophoretic separation. (Column 2, lines 56-66)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al by incorporating pH sensors to monitor buffer pH, as taught by Ishiwatari, because it would provide necessary data in processes sensitive to pH changes. An electronic pH sensor, such as that used by Ishiwatari, comprises an ion-selective electrode by definition. The electrode provides a variable signal dependent on the hydronium ion concentration, and is insensitive to other ions present in the solution.

Claim 4 lacks an inventive step under PCT Article 33(3) as being obvious over Ivory et al (US 5,298,143) in view of Koegler et al.

Ivory et al disclose a device suitable for determining the isoelectric point of a charged analyte (Figure 16), comprising: a chamber (53) comprising liquid inlets and outlets (86, 89, 321-345), and an electrode array (98, 422-424) isolated from the chamber and operative to establish an electric field gradient in the chamber. The chamber can be termed a "titration" chamber, if the apparatus is used in a titration method.

Ivory et al do not explicitly disclose a means for detecting analyte bands.

Koegler et al disclose an analyte band detector operative to generate a signal corresponding to detection of a band of analyte downstream of an electric field gradient in the titration chamber. (Figure 7; page 829, 1st paragraph)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al by incorporating an analyte band detector downstream of the electric field gradient in the titration chamber, as taught by Koegler et al, because it would allow identification of separated analytes.

Claims 5, 13, 51-53, 59-63, 66, and 67 lack inventive steps under PCT Article 33(3) as being obvious over Ivory et al (US 6,277,258).

Relevant to claims 5, 51, 59, and 67, Ivory et al disclose a device suitable for determining the isoelectric point of a charged analyte (Figures 3-6), comprising: a chamber (12) comprising liquid inlets and outlets (114 and 116), and an array of electrodes (22) isolated from the chamber and operative to establish an electric field gradient in the chamber. The chamber can be termed a "titration" chamber, if the apparatus is used in a titration method, or a "separation" chamber if used for a separation method.

Relevant to claim 13, Ivory et al disclose the use of a molecular sieve in the separation chamber to shift the location of a stationary focused band of analyte in the separation chamber for a given set of focusing conditions. (Column 6, lines 30-44)

Also relevant to claim 51, Ivory et al disclose an analyte band detector operative to generate positional signals corresponding to positions of a band of analyte in the titration chamber (Column 3, lines 32-37; Column 7, lines 8-12), and a processor operative to receive positional signals from the analyte band detector and to determine the magnitude of position change of an analyte band in the titration chamber in the course of titration. (Column 3, lines 32-37; Column 7, lines 8-12)

Relevant to claims 52, 60, and 61, Ivory et al disclose an electrode chamber comprising the electrodes. (14, Figure 6B)

Relevant to claims 53, 62, and 63, Ivory et al disclose the titration or separation chamber being separated from the electrode chamber by a permeable material. (16, Figure 6B)

Ivory et al do not explicitly disclose the use of a separation chamber and a titration chamber in fluid communication, both having electrodes as specified above for claims 5 and 59. (Claims 5, 59) They also do not explicitly disclose the use of a titration or separation chamber having a non-uniform cross-section channel (Claims 51, 59), but they suggest such a geometry. (Column 2, lines 28-34)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

Ivory et al by including a second device of identical structure in fluid communication with the first device, because it would allow for sequential analyses based on different parameters (e.g. one separation using molecular sieve) or prefractionation of more complex mixtures.

It would also have been obvious to modify the device of Ivory et al by configuring the titration or separation channel to have a non-uniform cross section, because it would provide an electric field gradient with a simpler electrode configuration.

Claims 6-8 and 10-12 lack inventive steps under PCT Article 33(3) as being obvious over the prior art as applied to claim 1 above and further in view of Ivory et al (US 6,277,258).

Ivory et al and Ishiwatari disclose a combination as described above in addressing Claim 1.

Neither Ivory et al nor Ishiwatari explicitly disclose a second chamber comprising the electrode array (Claim 6), the second chamber comprising a liquid inlet and outlet (Claim 7), the titration and second chambers separated by a permeable material (Claim 8), an analyte band detector (Claim 10), an analyte band detector in the first chamber (Claim 11), or molecular sieve in the titration chamber to shift the location of a stationary focused band of analyte for a given set of focusing conditions. (Claim 12)

Ivory et al (US 6,277,258) disclose a second chamber comprising the electrode array (14, Figure 6B), the second chamber comprising a liquid inlet and outlet (Figure 4, 215 and 217), the titration and second chambers separated by a permeable material (16, Figure 6B), an analyte band detector in the first chamber (Column 3, lines 32-37; Column 7, lines 8-12), and molecular sieve in the titration chamber to shift the location of a stationary focused band of analyte for a given set of focusing conditions. (Column 6, lines 30-44)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the combination of Ivory et al (US 5,298,143) and Ishiwatari by housing the electrode array in a chamber with a liquid inlet and outlet separated from the titration chamber by a permeable material, as taught by Ivory et al (US 6,277,258), because it would allow for circulation of a separate chilled electrode buffer to counteract Joule heating.

It would also have been obvious to modify the combination of Ivory et al (US 5,298,143) and Ishiwatari by including an analyte band detector in the titration chamber, as taught by Ivory et al (US 6,277,258), because it would allow for optimization of separation parameters during a run. (See US 6,277,258 Column 3, lines 32-37)

It would also have been obvious to modify the combination of Ivory et al (US 5,298,143) and Ishiwatari by including molecular sieve in the titration chamber, as taught by Ivory et al (US 6,277,258), because it would enhance some separations.

Claim 16 lacks an inventive step under PCT Article 33(3) as being obvious over Ivory et al (US 6,277,258) in view of Koegler et al.

Ivory et al disclose a device as described above in addressing claim 3.

Ivory et al do not explicitly disclose the use of a UV-Visible spectroscopy detector in their device.

Koegler et al disclose the use of a UV-Visible spectrophotometer as the detector in their apparatus. (Page 829, 1st paragraph)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al by using a UV-Visible spectrophotometer as a detector, because Ivory et al suggested optical detection (Column 3, lines 32-37) and it would provide an easily automated detection system.

Claims 17 and 18 lack inventive steps under PCT Article 33(3) as being obvious over the prior art as applied to claim 1 above and further in view of Nochumson et al.

Ivory et al and Ishiwatari disclose a combination as described above in addressing Claim 1.

Neither Ivory et al nor Ishiwatari explicitly disclose a mixing chamber with an inlet for receiving a liquid, an inlet for receiving a titrating solution, and an outlet connected to the inlet of the first chamber (Claim 17), or a mixing chamber comprising a static mixer. (Claim 18)

Nochumson et al disclose a device that requires the mixing of two fluids, comprising a static mixer with two inlets, and an outlet to flow the resulting mixed liquid to a subsequent location. (Figure 1, mixer prior to jacketed tank; Paragraph 0060)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the combination of Ivory et al and Ishiwatari by including a static mixer to mix two fluids prior to introduction to the subsequent chamber, as taught by Nochumson et al, because it would allow for efficient mixing of recirculating buffer from the titration chamber with modified buffer to effect a change in a separation parameter (e.g. pH, concentration), providing greater flexibility to the analysis.

Claim 19 lacks an inventive step under PCT Article 33(3) as being obvious over the prior art as applied to claim 1 above and further in view of Apffel.

Ivory et al and Ishiwatari disclose a combination as described above in addressing Claim 1.

Neither Ivory et al nor Ishiwatari explicitly disclose a device further comprising a dialyzer for dialyzing ions from a titrating solution to a flowing liquid or from a flowing liquid to a titrating solution.

Apffel discloses a device and method using on-line electromicrodialysis both before and after other separation methods, such as electrophoresis. (Figure 1; Column 6, line 62- Column 7, line12)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

combination of Ivory et al and Ishiwatari by including a dialyzer for dialyzing ions from a solution prior to further analysis (i.e. introduction to the titration chamber), as taught by Apffel, because it would remove salts that could increase solution conductivity and Joule heating.

Claims 43-45 lack inventive steps under PCT Article 33(3) as being obvious over Ivory et al (US 6,277,258) in view of Ishiwatari.

Relevant to claim 43, Ivory et al disclose a device suitable for determining the isoelectric point of a charged analyte (Figures 3-6), comprising: a chamber (12) comprising liquid inlets and outlets (114 and 116), and an array of electrodes (22) isolated from the chamber and operative to establish an electric field gradient in the chamber. The chamber can be termed a "titration" chamber, if used for a titration method.

Relevant to claim 44, Ivory et al disclose an electrode chamber comprising the electrodes. (14, Figure 6B)

Relevant to claim 45, Ivory et al disclose the titration chamber being separated from the electrode chamber by a permeable material. (16, Figure 6B)

Ivory et al do not explicitly disclose the use of a pH sensor positioned for exposure to the liquid introduced into the titration chamber or the use of a titration chamber with a non-uniform cross-section channel, though they suggest such a geometry. (Column 2, lines 28-34)

Ishiwatari discloses an electrophoretic device that uses pH sensors (8a and 8b, Figure 1) to detect the pH of the buffer solution that is used in the electrophoretic separation. (Column 2, lines 56-66)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al by incorporating pH sensors to monitor buffer pH, as taught by Ishiwatari, because it would provide necessary data in processes sensitive to pH changes.

It would also have been obvious to modify the device of Ivory et al by configuring the titration or separation channel to have a non-uniform cross section, because it would provide an electric field gradient with a simpler electrode configuration.

Claims 47-50 lack inventive steps under PCT Article 33(3) as being obvious over Ivory et al (US 6,277,258) in view of Ivory et al (US 5,298,143).

Relevant to claim 47, Ivory et al (US 6,277,258) disclose a device suitable for determining the isoelectric point of a charged analyte (Figures 3-6), comprising: a chamber (12) comprising liquid inlets and outlets (114 and 116), and an array of electrodes (22) isolated from the chamber and operative to establish an electric field gradient in the chamber. The chamber can be termed a "titration" chamber, if used for a titration method.

Relevant to claim 48, Ivory et al disclose an electrode chamber comprising the electrodes. (14, Figure 6B)

Relevant to claim 49, Ivory et al disclose the titration chamber being separated from the electrode chamber by a permeable material. (16, Figure 6B)

Ivory et al (US 6,277,258) do not explicitly disclose the use of a titration reservoir in fluid communication with the titration chamber, or the use of a titration chamber with a non-uniform cross-section channel, though they suggest such a geometry. (Column 2, lines 28-34) They also do not disclose an electrode chamber having a non-uniform cross-section flow channel.

Ivory et al (US 5,298,143) disclose a similar electrophoretic device that includes a reservoir in communication with the separation or titration chamber (Reservoir 135, Figure 12; Column 13, lines 12-23) and an electrode chamber with a non-uniform cross-section flow channel. (650, Figure 20)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al (US 6,277,258) by adding an external reservoir of fluid for circulation into the separation chamber, as taught by Ivory et al (US 5,298,143), because it would allow for more efficient cooling of the fluid.

It would also have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al (US 6,277,258) by providing an electrode chamber with a non-uniform cross-section flow channel, as taught by Ivory et al (US 5,298,143), because it would allow for an electric field gradient in a separation chamber of uniform cross-section with only two electrodes.

It would also have been obvious to modify the device of Ivory et al (US 6,277,258) by configuring the titration or separation channel to have a non-uniform cross section, because it would provide an electric field gradient with a simpler electrode configuration.

Claims 55-58 lack inventive steps under PCT Article 33(3) as being obvious over Ivory et al (US 6,277,258) in view of Koegler et al.

Relevant to claim 55, Ivory et al (US 6,277,258) disclose a device suitable for determining the isoelectric point of a charged analyte (Figures 3-6), comprising: a chamber (12) comprising liquid inlets and outlets (114 and 116), an array of electrodes (22) isolated from the chamber and operative to establish an electric field gradient in the chamber, and an analyte band detector operative to generate positional signals corresponding to positions of a band of analyte in the titration chamber. (Column 3, lines 32-37; Column 7, lines 8-12) The chamber can be termed a "titration" chamber, if used for a titration method.

Relevant to claim 56, Ivory et al disclose an electrode chamber comprising the electrodes. (14, Figure 6B)

Relevant to claim 57, Ivory et al disclose the titration chamber being separated from the electrode chamber by a permeable material. (16, Figure 6B)

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Ivory et al (US 6,277,258) do not explicitly disclose the use of an analyte band detector positioned downstream of an electric field gradient in the titration chamber, or the use of a titration chamber with a non-uniform cross-section channel, though they suggest such a geometry. (Column 2, lines 28-34)

Koegler et al disclose an analyte band detector operative to generate positional signals corresponding to positions of a band of analyte downstream of an electric field gradient in the titration chamber. (Figure 7; Page 829, 1st paragraph) They also disclose a device with an electrode chamber having a non-uniform cross-section. (Figure 7; Equipment section)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al (US 6,277,258) by positioning the detector downstream of an electric field gradient in the titration chamber, as taught by Koegler et al, because it would simplify device construction.

It would also have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al (US 6,277,258) by providing an electrode chamber with a non-uniform cross-section flow channel, as taught by Koegler et al, because it would allow for an electric field gradient in a separation chamber of uniform cross-section with only two electrodes.

It would also have been obvious to modify the device of Ivory et al by configuring the titration or separation channel to have a non-uniform cross section, because it would provide an electric field gradient with a simpler electrode configuration.

Claims 76-79 lack inventive steps under PCT Article 33(3) as being obvious over Ivory et al (US 6,277,258) in view of Ivory et al (US 5,298,143).

Relevant to claim 76, Ivory et al (US 6,277,258) disclose a device suitable for determining the isoelectric point of a charged analyte (Figures 3-6), comprising: a separation chamber (12) comprising liquid inlets and outlets (114 and 116), an electrode chamber (14) of uniform depth, more than two electrodes (22) in the electrode chamber operative to establish an electric field gradient in the separation chamber, an analyte band detector operative to generate positional signals corresponding to positions of a band of analyte in the titration chamber (Column 3, lines 32-37; Column 7, lines 8-12), and a processor operative to receive positional signals from the analyte band detector and to determine the magnitude of position change of an analyte band in the titration chamber in the course of titration. (Column 3, lines 32-37; Column 7, lines 8-12) The separation chamber can be termed a "titration" chamber, if used for a titration method.

Relevant to claim 77, Ivory et al disclose the titration or separation chamber being separated from the electrode chamber by a permeable material. (16, Figure 6B)

Relevant to claim 78, Ivory et al disclose the titration chamber having a uniform cross-section flow channel. (Figure 3A-3E)

Ivory et al (US 6,277,258) do not explicitly disclose an electrode chamber having non-uniform width, nor do they disclose the use of a titration chamber with a non-uniform cross-section channel, though they suggest such a geometry. (Column 2, lines 28-34)

Ivory et al (US 5,298,143) disclose an electrode chamber having non-uniform width. (650, Figure 20)

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al (US 6,277,258) by configuring the electrode chamber to have a uniform depth and non-uniform width, as taught by Ivory et al (US 5,298,143), because it would allow the use of only two electrodes to provide the electric field gradient.

It would also have been obvious to modify the device of Ivory et al by configuring the titration or separation channel to have a non-uniform cross section, because it would provide an electric field gradient with a simpler electrode configuration.

Claims 84-97 (including both claims 85) lack inventive steps under PCT Article 33(3) as being obvious over Ivory et al (US 6,277,258) in view of Ivory et al (US 5,298,143).

Relevant to claims 84, 85a, 90, and 91, Ivory et al (US 6,277,258) disclose a device suitable for determining the isoelectric point of a charged analyte (Figures 3-6), comprising: a separation chamber (12) comprising liquid inlets and outlets (114 and 116), an electrode chamber with uniform depth (14, Figure 6B), and plural electrodes (22) in the electrode chamber operative to establish an electric field gradient in the separation chamber. The separation chamber can be termed a "titration" chamber, if the apparatus is used in a titration method.

Relevant to claims 85b, 86, 92, and 93, Ivory et al disclose the separation/titration chamber being separated from the electrode chamber by a permeable material. (16, Figure 6B)

Relevant to claims 89 and 97, Ivory et al disclose an electrode array (22) in the electrode chamber.

Also relevant to claim 90, Ivory et al disclose an electrode array (22) isolated from the separation chamber and operative to establish an electric field gradient in the separation chamber.

Ivory et al do not explicitly disclose the use of a separation chamber and a titration chamber in fluid communication, both having electrodes as specified above for claims 84 and 90. They also do not disclose an electrode chamber having non-uniform width or cross-section. (Claims 84, 87, 90, and 94) They also do not explicitly disclose the use of a titration or separation chamber having a non-uniform cross-section channel (Claims 88, 95, and 96), but they suggest such a geometry. (Column 2, lines 28-34)

Ivory et al (US 5,298,143) disclose a similar device with an electrode chamber that has non-uniform width. (650, Figure 20)

Relevant to claims 84-97, it would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al (US 6,277,258) by providing an electrode chamber with non-uniform width, as taught by Ivory et al (US 5,298,143), because it would allow the use of only two electrodes to provide the electric field gradient.

Addressing claims 84-87, 89-94, and 97, it would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al (US 6,277,258) by including a second device of identical structure in fluid

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communication with the first device, because it would allow for sequential analyses based on different parameters (e.g. one separation using molecular sieve) or prefractionation of more complex mixtures.

Addressing claims 88, 95, and 96, it would also have been obvious to modify the device of Ivory et al (US 6,277,258) by configuring the titration or separation channel to have a non-uniform cross section, because it could provide an electric field gradient with a simpler electrode configuration.

Claim 71 lacks an inventive step under PCT Article 33(3) as being obvious over the prior art as applied above in addressing claim 68 and further in view of Ivory et al (US 6,277,258).

Ivory et al (US 5,298,143) and Ishiwatari disclose a combination as described above in addressing claim 68.

Neither Ivory et al (US 5,298,143) nor Ishiwatari explicitly disclose a titration chamber having a non-uniform cross-section flow channel.

Ivory et al (US 6,277,258) do not explicitly disclose the use of a titration chamber with a non-uniform cross-section channel, though they suggest such a geometry. (Column 2, lines 28-34)

It would also have been obvious to modify the combination of Ivory et al (US 5,298,143) and Ishiwatari by configuring the titration or separation channel to have a non-uniform cross section, as suggested by Ivory et al (US 6,277,258), because it would provide an electric field gradient with a simpler electrode configuration.

Claim 75 lacks an inventive step under PCT Article 33(3) as being obvious over Ivory et al (US 5,298,143) in view of Ivory et al (US 6,277,258).

Ivory et al (US 5,298,143) disclose a device as described above in addressing claim 72.

Ivory et al (US 5,298,143) do not explicitly disclose a titration chamber having a non-uniform cross-section flow channel.

Ivory et al (US 6,277,258) do not explicitly disclose the use of a titration chamber with a non-uniform cross-section channel, though they suggest such a geometry. (Column 2, lines 28-34)

It would also have been obvious to modify the device of Ivory et al (US 5,298,143) by configuring the titration or separation channel to have a non-uniform cross section, as suggested by Ivory et al (US 6,277,258), because it would provide an electric field gradient with a simpler electrode configuration.

Claim 83 lacks an inventive step under PCT Article 33(3) as being obvious over Koegler et al in view of Ivory et al (US 6,277,258).

Koegler et al disclose a device as described above in addressing claim 68.

Koegler et al do not explicitly disclose a titration chamber having a non-uniform cross-section flow channel.

Ivory et al (US 6,277,258) do not explicitly disclose the use of a titration chamber with a non-uniform cross-section channel, though they suggest such a geometry. (Column 2, lines 28-34)

It would also have been obvious to modify the device of Koegler et al by configuring the titration or separation channel to have a non-uniform cross section, as suggested by Ivory et al (US 6,277,258), because it would provide an electric field gradient with a simpler electrode configuration.

Claim 46 lacks an inventive step under PCT Article 33(3) as being obvious over the prior art as applied above in addressing claim 43 and further in view of Ivory et al (US 5,298,143).

Ivory et al (US 6,277,258) and Ishiwatari disclose a combination as described above in addressing claim 43.

Neither Ivory et al (US 6,277,258) nor Ishiwatari explicitly disclose the use of an electrode chamber with a non-uniform cross-section flow channel.

Ivory et al (US 5,298,143) disclose an electrode chamber (650, Figure 20) having a non-uniform cross-section flow channel.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the combination of Ivory et al (US 6,277,258) and Ishiwatari by providing an electrode chamber with a non-uniform cross-section flow channel, as taught by Ivory et al (US 5,298,143), because it would allow for an electric field gradient in a separation chamber of uniform cross-section with only two electrodes.

Claims 54, 64, and 65 lack inventive steps under PCT Article 33(3) as being obvious over Ivory et al (US 6,277,258) in view of Ivory et al (US 5,298,143).

Ivory et al (US 6,277,258) discloses a device as described above in addressing claims 51 and 55.

Ivory et al (US 6,277,258) do not explicitly disclose the use of an electrode chamber with a non-uniform cross-section flow channel.

Ivory et al (US 5,298,143) disclose an electrode chamber (650, Figure 20) having a non-uniform cross-section flow channel.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the device of Ivory et al (US 6,277,258) by providing an electrode chamber with a non-uniform cross-section flow channel, as taught by Ivory et al (US 5,298,143), because it would allow for an electric field gradient in a separation chamber of uniform cross-section with only two

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electrodes.

Claims 20-42 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a method for determining the isoelectric point of a charged analyte comprising: focusing the analyte in a flowing liquid an electric field gradient; changing the pH of the flowing liquid in order to change the position of the focused band; obtaining pH and corresponding position data for the analyte, comprising determining the pH of the flowing liquid and the corresponding position of the focused band of the charged analyte at a plurality of band positions within the electric field gradient; and determining the isoelectric point of the analyte based on the PH and corresponding position data.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;
(ii) the claim is cancelled;
(iii) the claim is new;
(iv) the claim replaces one or more claims as filed;
(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under Article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see the *PCT Applicant's Guide*, Volume II.